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Soluble guanylyl cyclase: A novel target for the treatment of vascular cognitive impairment?

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ABSTRACT

Vascular cognitive impairment (VCI) describes neurodegenerative disorders characterized by a vascular component. Pathologically, it involves decreased cerebral blood flow (CBF), white matter lesions, endothelial dysfunction, and blood-brain barrier (BBB) impairments. Molecularly, oxidative stress and inflammation are two of the major underlying mechanisms. Nitric oxide (NO) physiologically stimulates soluble guanylate cyclase (sGC) to induce cGMP production. However, under pathological conditions, NO seems to be at the basis of oxidative stress and inflammation, leading to a decrease in sGC activity and expression. The native form of sGC needs a ferrous heme group bound in order to be sensitive to NO (Fe(II)sGC). Oxidation of sGC leads to the conversion of ferrous to ferric heme (Fe(III)sGC) and even heme-loss (apo-sGC). Both Fe(III)sGC and apo-sGC are insensitive to NO, and the enzyme is therefore inactive. sGC activity can be enhanced either by targeting the NO-sensitive native sGC (Fe(II)sGC), or the inactive, oxidized sGC (Fe(III)sGC) and the heme-free apo-sGC. For this purpose, sGC stimulators acting on Fe(II)sGC and sGC activators acting on Fe(III)sGC/apo-sGC have been developed. These sGC agonists have shown their efficacy in cardiovascular diseases by restoring the physiological and protective functions of the NO-sGC-cGMP pathway, including the reduction of oxidative stress and inflammation, and improvement of vascular functioning. Yet, only very little research has been performed within the cerebrovascular system and VCI pathology when focusing on sGC modulation and its potential protective mechanisms on vascular and neural function. Therefore, within this review, the potential of sGC as a target for treating VCI is highlighted.

The term vascular cognitive impairment (VCI) was introduced in the early 2000 s to encompass all types of cognitive and neurodegenerative disorders with cognitive impairments related to vascular components [1]. VCI is a comprehensive term that includes various subtypes,

Abbreviations: AD, Alzheimer's Disease; apo-sGC, inactive heme-fee sGC; BBB, blood-brain barrier; CADASIL, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; cAMP, cyclic adenosine monophosphate; CBF, cerebral blood flow; cGMP, cyclic guanosine monophosphate; CNG, cyclic nucleotide gated; CNS, central nervous system; cSVD, cerebral small vessel disease; Cyb5R3, cytochrome b5 reductase 3; FeIIsGC, NO-sensitive native sGC; FeIIIsGC, inactive oxidized sGC; GFAP, glial fibrillary acidic protein; GTP, guanosine 5'-triphosphate; HCN, hyperpolarization-activated cyclic nucleotide-modulated; H-NOX, N-heme-nitric oxide binding domain; Hsp90, 90 kDa heat shock protein; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin 6; LPS, lipopolysaccharide; LTP, long-term potentiation; MELAS, Mitochondrial Encephalopathy Lactic Acidosis and Stroke-like episodes; MYOCD, myocardin; NO, nitric oxide; NOS, nitric oxide synthase; NVU, neurovascular unit; PDE, cyclic nucleotide phosphodiesterase; PKG, protein kinase G; sGC, soluble guanylyl cyclase; SOD, superoxide dismutase; SRF, serum response factor; TNF-α, tumor necrosis factor α; VASP, vasodilator-stimulated phosphoprotein; VCI, vascular cognitive impairment; VSMC, vascular smooth muscle cell.

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typically defined by two criteria: I) a clinically significant deficit in at least one cognitive domain causing the severe disruption of daily living activities, and II) imaging evidence of cerebrovascular pathology [2]. Within major VCI, there are four different subtypes called I) post-stroke dementia, II) subcortical ischemic vascular dementia, III) multi-infarct dementia, and IV) mixed dementia [3]. The primary cause of VCI is cerebral small vessel disease (cSVD) [4], although large vessel disease is also prevalent.

The pathobiology of VCI is even more variable, yet can still be categorized into key processes: white matter lesions, reduced cerebral blood flow (CBF), hemorrhages, and infarcts [3,5]. Most of these pathological processes in VCI can be attributed to cSVD pathology. cSVD pathology is well-defined due to its hereditary components, although sporadic cSVD also exists and is actually the most prevalent form of cSVD. cSVD can encompass athero- and arteriolosclerosis, microbleeds, small infarcts, and cerebral amyloid angiopathy [3,6]. However, many of these pathological features can also be observed in other dementias [7,8]. Of note, stroke can also be a cause for the manifestation of VCI, but due to the different initial underlying pathological processes, this is outside the scope of this review.

Underlying molecular mechanisms of VCI include inflammation, oxidative stress, endothelial dysfunction, and blood-brain barrier (BBB) leakage [9]. In the physiological maintenance of (cerebral)vascular health, which includes healthy endothelial functioning and an intact BBB, the nitric oxide (NO) – soluble guanylate cyclase (sGC) - 3',5'-cyclic guanosine monophosphate (cGMP) pathway plays a significant role. Remarkably, this pathway is also involved in neuronal processes related to memory formation. Pathologically, there are strong indications that this pathway can be disrupted in cardiovascular disease, with sGC function appearing particularly sensitive to pathological processes such as oxidative stress [10]. However, the central role of sGC in VCI pathology and its potential as a pharmacological target for the treatment of VCI has not been extensively discussed in current literature. Consequently, this review aims to provide an overview of the current knowledge on physiological NO-sGC-cGMP signaling in the cardiovascular system and central nervous system (CNS), and the interface between these two at the level of the neurovascular unit (NVU). Additionally, it summarizes the molecular pathological processes associated with VCI, highlighting the dysfunction of sGC. Finally, the review discusses pharmacological targeting of sGC as a treatment strategy for VCI.

1. Physiological NO-sGC-cGMP signaling

1.1. Nitric oxide, soluble guanylyl cyclase, and the production of cGMP

NO is a gaseous, lipid-soluble molecule that can freely cross cellular membranes, and in 1998 Robert Furchgott, Louis Ignarro and Ferid Murad were awarded the Nobel Prize for discovering its role as a signaling molecule in the cardiovascular system. Today, we understand that NO is involved in a variety of important physiological processes, including fibrotic remodeling, neuronal functioning, and inflammation (for a review, see [11]). NO can be produced by several nitric oxide synthases (NOS): constitutive cNOS (endothelial eNOS and neuronal nNOS) and inducible iNOS (found in a wide range of tissues). The expression and activation of iNOS are calcium (Ca^{2+})-independent, and are induced after exposure to lipopolysaccharide (LPS) or inflammatory cytokines such as interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor α (TNF-α) [12,13]. In contrast, cNOS activation is Ca^{2+} dependent and is responsible for the production of physiological NO levels [14].

Focusing on the physiological conditions, NO produced by cNOS can stimulate the protein sGC. sGC (also known as NO-sensitive GC, NO-GC) is a heterodimeric enzyme consisting of a larger α-subunit and a smaller, heme-binding β-subunit [15,16]. Currently, four different subunits are known for sGC: α1, α2, β1 and β2 [17–21]. Each subunit possesses an N-terminal regulatory domain and C-terminal catalytic domain, homologous to both particulate guanylyl cyclase and adenylate cyclase [22]. Two main sGC isoforms have been identified: NO-GC1, composed of α1/β1 subunits, and NO-GC2, consisting of α2/β1 subunits [23]. Enzymes containing the β2 subunit appear to lack enzymatic activity [24,25]. sGC activity and sensitivity to NO depend on the association between a prosthetic heme group and the histidine-105 (His-105) residue of the β-subunit on the so-called N-heme-nitric oxide binding domain (H-NOX) [26]. Upon binding of NO to the ferrous heme group bound to the H-NOX domain, the bond between the heme and the His-105 is broken, resulting in a several hundred-fold increase in sGC activity above basal levels [27]. Consequently, sGC catalyzes the conversion of guanosine 5'-triphosphate (GTP) to cGMP through a cyclisation reaction [28].

Interestingly, oxidation of the heme group bound to sGC causes a transition from the ferrous to the ferric state (from Fe^{2+} to Fe^{3+}). Ligands such as NO do not bind effectively to this ferric state, rendering NO incapable of activating sGC [29,30]. Furthermore, oxidation of heme-bound sGC can lead to the complete loss of the heme group (resulting in apo-sGC) [31,32], triggering ubiquitination and proteasomal degradation of the enzyme [33]. Several studies have shown that ferrous-bound, native sGC (Fe(II)sGC) is more stable than ferric-bound sGC (Fe(III)sGC), and Fe(III)sGC loses its heme group much more readily than Fe(II)sGC does [34,35], likely due to oxidation-induced conformational changes that expose the heme-binding pocket, thereby facilitating heme-loss [36]. This results in an inactive and heme-free sGC enzyme (apo-sGC) that cannot bind NO and, as a result, does not catalyze cGMP formation. Recently research has shown that smooth muscle cells express cytochrome b5 reductase 3 (Cyb5R3), a flavoprotein capable of directly reducing oxidized ferric-sGC to its native (ferrous) form [37,38]. Thus, Cyb5R3 might provide an endogenous protection against oxidative stress and endothelial dysfunction in the vasculature.

cGMP is an intracellular signaling molecule in a range of cell types, and its response is regulated endogenously through the rate of production by sGC, or the rate of degradation by cyclic nucleotide phosphodiesterases (PDEs). Currently, 11 families of PDE enzymes have been identified, of which PDE5, 6 and 9 specifically hydrolyze cGMP while PDE4, 7 and 8 specifically hydrolyze cyclic adenosine monophosphate (cAMP), and PDE1, 2, 3, 10 and 11 can hydrolyze both cyclic nucleotides [39]. Importantly, each PDE family comprises multiple genes, splice variants, and isoforms. For the dual substrate PDE1, 2, 3, 10 and 11, different isoforms can still exhibit varying affinities for cyclic nucleotides; e.g. PDE1A, PDE1B and PDE10A have a higher affinity for cGMP [39]. The complexity of the PDE enzyme family enables PDEs to play a key role in the temporal and spatial organization of cGMP signaling, shaping NO-sGC induced cGMP signaling into distinct compartments and microdomains within a cell [40]. This organization and subcellular localization significantly diversify the cellular effects of cGMP, resulting in a complex signaling system [41].

Activation of the cGMP signaling pathway can activate protein kinase G (PKG; see Fig. 1). Two distinct PKG genes have been identified: PKG-I and PKG-II [42]. PKG-I is widely expressed in tissues including cerebellum, smooth muscle cells, and platelets. Due to alternative splicing, PKG-I can be subdivided into PKG-Iα and PKG-Iβ isoforms, each with different binding affinities for cGMP and distinct expression profiles in tissues [43]. PKG-Iα has a higher affinity for cGMP and is primarily expressed in the cerebellum, adrenal glands, kidneys, and the vasculature. PKG-Iβ has a lower affinity for cGMP and is predominantly expressed in the hippocampus, uterus, smooth muscle, and platelets [42,43]. PKG-II closely resembles PKG-I, with the exception of its N-terminus, which dictates cellular localization of the enzyme: PKG-I is soluble, whereas PKG-II is membrane bound. PKG-II is most abundant in the brain and intestine, while also present in the kidneys, lung, and bone [42,43]. PKG-II is notably absent from the vasculature, and has a considerably lower cGMP binding affinity compared to PKG-I [44]. Upon activation by cGMP, PKG can phosphorylate various downstream

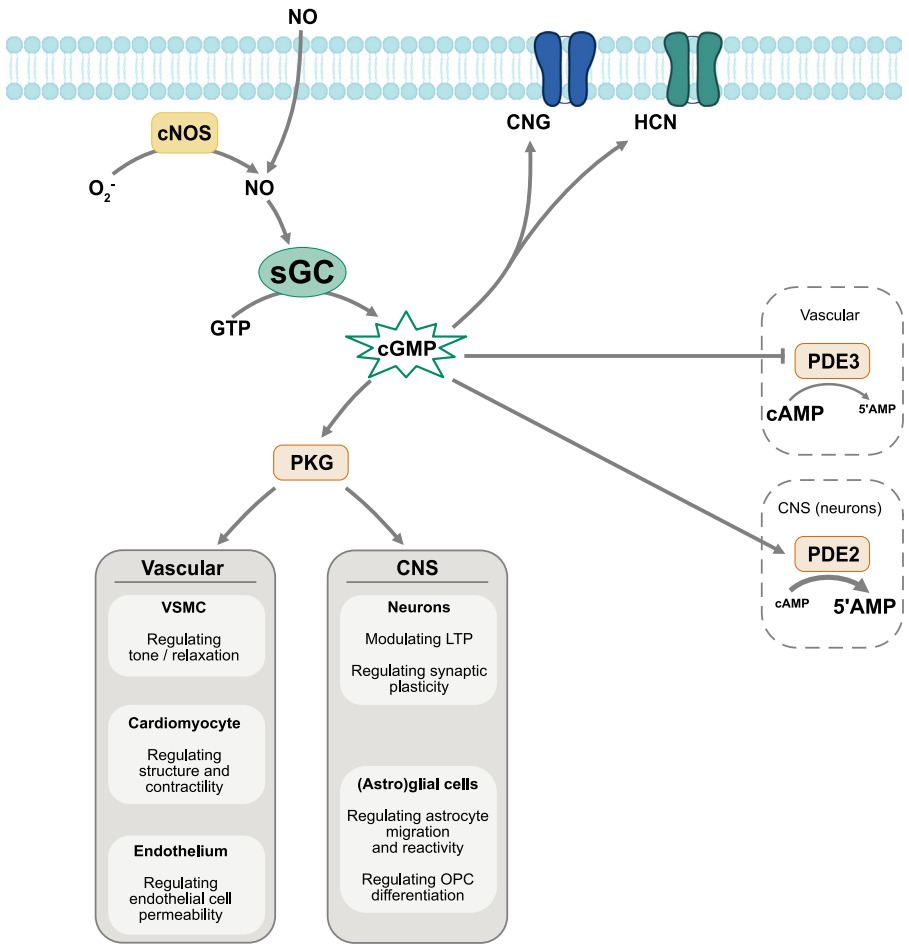

Fig. 1. Schematic overview of the main sGC-cGMP signaling pathways in vascular systems and the CNS. sGC activity is stimulated by NO, derived from NOS activity either within the cell, or from surrounding cells. cGMP formation catalyzed by sGC activity results in the activation of PKG, which can have many different effects through effector molecules, depending on the cell type. cGMP itself can also have a direct effect on the polarization of CNG and HCN channels, and within blood vessels and cardiomyocytes, cGMP can directly inhibit PDE activity. This results in prolonged cAMP signaling within these cellular systems. cAMP: cyclic adenosine monophosphate, cGMP: cyclic guanosine monophosphate, CNG: cyclic nucleotide gated ion channel, cNOS: constitutive nitric oxide synthase, CNS: central nervous system, GTP: guanosine triphosphate, HCN: hyperpolarization-activated cyclic nucleotide mediated ion channel, NO: nitric oxide, O_2^-: superoxide anion, PDE: cyclic nucleotide phosphodiesterase, sGC: soluble guanylyl cyclase, VSMC: vascular smooth muscle cell, 5'-AMP: 5'-adenosine monophosphate.

targets, depending on the cellular compartmentalization and localization. cGMP signaling plays distinct roles in the vascular system and brain (Fig. 1). Therefore, this review will primarily focus on cellular NO-cGMP signaling in the cardiovascular system, the central nervous system (CNS), and the interface between these two systems, i.e. the neurovascular unit, in physiological conditions and in the context of VCI.

Importantly, GMP not only interacts with PKG but can also bind to other targets. cGMP can directly influence the cell by acting on cyclic nucleotide gated (CNG) ion channels and hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels (Fig. 1). CNG channels are ligand-gated cation channels with the ligand-binding site located on the cytoplasmic side of the cell membrane, allowing the influx of mainly Na^+ and Ca^{2+} upon binding of cyclic nucleotides [45]. Additionally, cGMP can directly bind to PDEs and modulate PDE activity. This has been demonstrated for PDE2 in neuronal cells which could be activated by cGMP, and PDE3 which could be inhibited by cGMP in vascular cells (Fig. 1) [46–50]. Thus, cGMP could lead to reduced cAMP levels in neuronal cells via PDE2 activation, while cGMP-mediated PDE3 inhibition could lead to enhanced cAMP formation in blood vessels and cardiomyocytes. This interaction between cGMP and PDEs might diminish or amplify the effects of cGMP signaling by connecting cGMP pools with cAMP pools [51,52]. This results in cell type specific modulation of cGMP signaling. Clearly, further research is needed to clarify the impact of this interaction in CNS diseases and cognitive function. In the following sections, the main physiological pathways and effects of NO-sGC-cGMP signaling in the vascular system and CNS will be reviewed more in depth (also see Fig. 1).

1.2. NO-sGC-cGMP signaling in the vascular system

In the vascular system, cGMP signaling is known to affect vascular smooth muscle cells (VSMCs). The majority of the NO-sGC-cGMP effects within these cells derive from subsequent PKG-I activation [53] (Fig. 1). Yet this signaling first starts in endothelial cells, where eNOS is expressed and NO is produced, which can then diffuse intercellularly. Remarkably, NO can also impact sGC signaling within the endothelium itself, by regulating endothelial cell permeability and enhancing endothelial barrier functioning [54–56]. Activation of PKG-I can elicit a wide range of effects, with its influence on VSMC tone being one of the most well-known (Fig. 1). The NO-sGC-cGMP cascade can induce VSMC relaxation via both PKG-Iα and PKG-Iβ mechanisms [57]. VSMC tone regulation via PKG-Iα involves interaction with myosin light-chain phosphatase [58], phosphorylation of phospholamban [59], phosphorylation of calcium-activated potassium channel [60], phosphorylation of regulator of G-protein signaling-2 [61], and interaction with RhoA [62]. cGMP-induced activation of PKG-Iβ results in the phosphorylation of IP_3

receptor associated cGMP kinase substrate, which in turn can inhibit the IP_3 induced release of Ca^{2+} from the endoplasmic reticulum via the IP_3 receptor, leading to muscle relaxation [63–65]. Additionally, PKG-Iα phosphorylates vasodilator-stimulated phosphoprotein (VASP), thereby regulating not only cardiomyocyte structure and contractility, but also influencing endothelial cell permeability and VSMC tone [66,67].

1.3. NO-sGC-cGMP signaling in the CNS system

One of the best-known effects of the NO-sGC-cGMP signaling pathway in the CNS is its role in modulating synaptic plasticity and memory processes. Long-term potentiation (LTP) has been widely accepted as the primary cellular model that describes the neurophysiological process underlying memory formation [68,69]. LTP involves a complex interplay of pre-synaptic and post-synaptic signaling cascades that enhance the efficiency of signal transduction. This includes increased neurotransmitter release and synaptic and cellular plasticity, such as increased insertion of receptors in pre- and post-synaptic membranes, strengthened synapse integrity, and enhanced neurite outgrowth and synapse formation [70]. Studies have identified the involvement of the NO-sGC-cGMP pathway in AMPA receptor dynamics [71], the maintenance of LTP [72,73], effects on pre-synaptic plasticity [74], and pre-synaptic neurotransmitter release [75]. Additionally, the interplay between pre-and post-synaptic NO-sGC-cGMP signaling was found to be crucial for LTP [76]. In this interplay, the two distinct subunit-dependent isoforms of sGC appear to play a vital role: despite an equal distribution of the isoforms within the brain, NO-GC1 (α1/β1) is likely involved in pre-synaptic functioning, whereas NO-GC2 (α2/β1) has been associated with post-synaptic proteins and is important for post-synaptic functioning [23,24,76].

In addition to its effects on PKG, cGMP can also directly influence the neuron by acting on CNG ion channels and HCN channels (Fig. 1). CNG channels allow the influx of primarily Na^+ and Ca^{2+} upon binding of cyclic nucleotides [45] and their expression in the brain is widespread [77], including the hippocampus [78]. HCN channels are also present in the brain, with the voltage-dependence of HCN1 channels in the hippocampus being modulated by both pre-synaptic NO-sGC1-cGMP signaling, and post-synaptic NO-sGC2-cGMP signaling, promoting HCN channel activity and depolarization [79,80]. Pre-synaptically this leads to induced glutamate release [79], whereas post-synaptically, the depolarization of HCN channels has been found to modulate and promote depolarization of NMDA receptors, thereby contributing to hippocampal LTP [80].

Next to its impact on neurons, the NO-sGC-cGMP pathway also plays a crucial role in the functioning of glial cells. Within astrocytes, which are a part of the tripartite synapse, it has been observed that NO-dependent increases in cGMP levels lead to actin remodeling and subsequent morphological changes, and promoted astrocyte migration [81]. Furthermore, the expression of glial fibrillary acidic protein (GFAP) is regulated by NO-sGC-cGMP signaling pathways [82], which is likely connected to the iNOS-dependent control of astrocyte reactivity and astrogliosis in the presence of pro-inflammatory cytokines such as IL-1β, TNF-α, and interleukin-6 (IL-6) [83,84]. Moreover, enhancing sGC-cGMP-PKG signaling through the physiological NO donor S-Nitrosoglutathione increases the production of ciliary neurotrophic factor by astrocytes. This, in turn, promotes the differentiation of oligodendrocyte precursor cells into myelinating oligodendrocytes [85]. The sGC signaling in oligodendrocytes is associated with activity-dependent adaptive myelination, which is part of the processes involved in learning and memory. *In vivo* studies have shown that increasing intracellular cGMP levels by inhibiting PDE5 (using sildenafil) improves myelin structures. However, it remains to be determined whether this effect is a direct result of the modulation of oligodendrocyte lineage cells and whether it persists when stimulating the NO-sGC-cGMP pathway [86]. In microglia, the role of the NO-sGC-cGMP signaling pathway is still a subject of debate. Similar to astrocytes, elevating cGMP signaling in microglia and macrophages leads to a reorganization of the actin cytoskeleton, resulting in morphological changes associated with increased phagocytic activity [81,87,88]. Interestingly, the sGC-cGMP pathway has been shown to negatively regulate the phagocytic activity of microglia [89]. In addition, while increasing the NO concentration in microglia-neuron cocultures has been found to enhance the phagocytic capacity of microglia for clearing apoptotic neuron bodies, this effect appears to be independent of the sGC-cGMP pathway[89]. This suggests that the observed rise in phagocytic activity resulting from higher cGMP levels cannot be ascribed to sGC; instead, it likely involves other pathways, such as particulate guanylyl cyclase signaling.

1.4. NO-sGC-cGMP signaling in the neurovascular unit

Regulation of cerebral vascular tone and neuronal function involves signaling cascades in both blood vessels and neurons. Therefore, it is crucial to understand cyclic nucleotide and cGMP signaling in both compartments. Moreover, both systems influence each other, known as neurovascular coupling. Neurovascular coupling refers to the synergy and communication between the cerebral microcirculation and neuronal activity, occurring at the level of the BBB and the NVU. Essentially, it is the ability of cerebral small vessels to adjust local blood flow in accordance with neuronal activity. The NVU forms the foundation of neurovascular coupling, with a focus on the microvascular level at arterioles and capillaries. The NVU in arterioles consists of endothelial cells, VSMCs, astrocytic end feet and neural processes (Fig. 2). In contrast, the NVU in capillaries lacks VSMCs but instead contains pericytes embedded in a basement membrane [90].

Neurons: From a neuronal perspective, NO derived from nNOS and produced upon activation of NMDA receptors in hippocampal glutamatergic neurons was shown to diffuse from neurons into the associated arterioles [91] (Fig. 2). The diffusion of NO resulted in a local increase in CBF and subsequently, a rise in O_2 tension due to elevated sGC activity in the local cerebral vasculature (Fig. 2). This mechanism did not rely on eNOS and was likely astrocyte-independent [92]. In the cerebellum, a similar vasodilatory response has been suggested to be mediated by GABAergic cerebellar stellate cells through extrasynaptic NMDA receptor activation, subsequent nNOS activation, NO release, and diffusion into nearby microvessels [93]. Overall, neuronal NO-sGC-cGMP-dependent contributions to the regulation of CBF seem mostly confined to the arterioles (Fig. 2).

Astrocytes: From the perspective of astrocytes, their role in the NVU seems to be limited to capillaries. This astrocyte-mediated vasodilatory mechanism in capillaries is independent of NO. Instead, it involves Ca^{2+} entry trough ATP-gated channels in astrocytes, which induces a transient increase in intracellular Ca^{2+} levels. This increase activates phospholipase 2D and diacylglycerol kinase, leading to the subsequent synthesis of arachidonic acid metabolites [94]. This contrasts with the neuronal NO-sGC-cGMP dependent mechanism in arterioles.

Pericytes: The role of pericytes in the NVU also appears to be restricted to capillaries. However, in contrast to astrocytes, the pericyte-mediated vasodilatory mechanism has been carefully suggested to be NO-sGC-cGMP dependent, yet the mechanism itself has not been clearly described [95].

A crucial function of the NVU is the formation of the BBB, which is formed by endothelial cells tightly sealed together through tight-junction protein. The exchange of substances between the blood and the brain relies on molecule-specific transporters and endothelial transcytosis. When considering NO-sGC-cGMP signaling, it has been observed that NO-dependent cGMP signaling in brain capillary endothelial cells can lead to the phosphorylation of VASP at Ser239. This phosphorylation is suggested to influence tight junction formation and endothelial permeability [96]. Additionally, NO-sGC-cGMP signaling was found to modulate the infiltration of T-cells by reducing their adhesion to the brain endothelium [97]. However, the understanding of NO-sGC-cGMP signaling in BBB maintenance under homeostatic

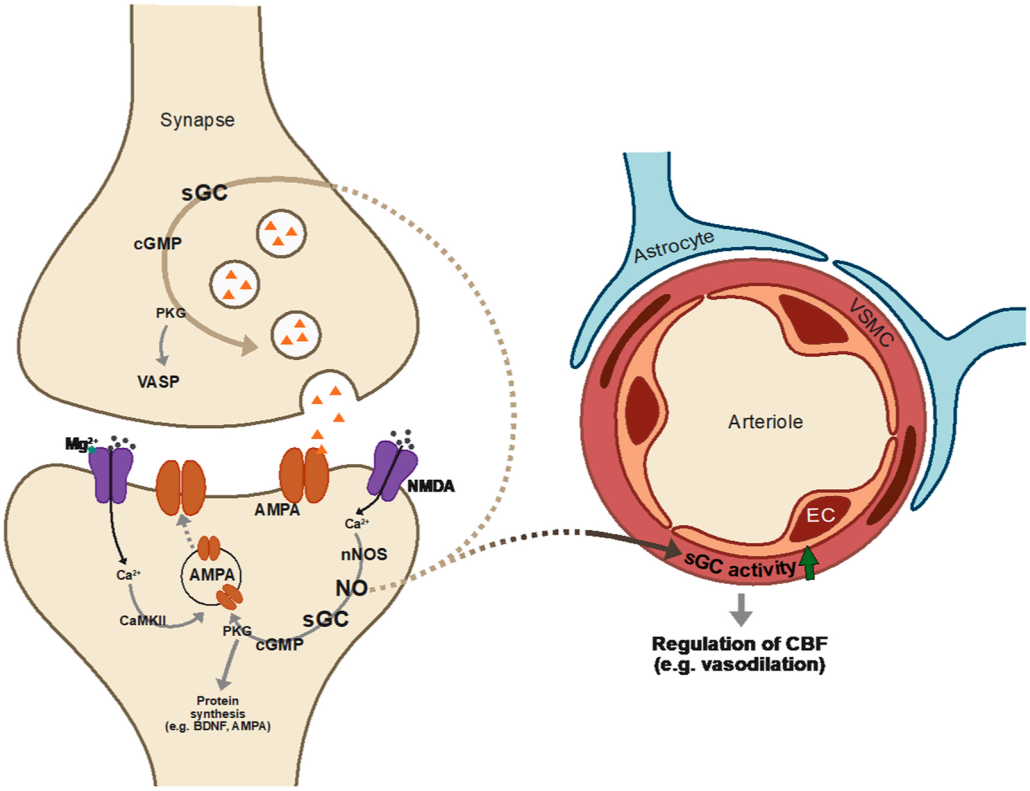

Fig. 2. Schematic overview of the neuronal effects of NO-sGC-cGMP signaling on CBF regulation through vasodilation of cerebral arterioles. Within the neuron, activation of NMDA receptors leads to the intracellular increase in Ca^{2+}, thereby activating the activity of nNOS. The NO produced by nNOS can then diffuse to nearby arterioles in which it stimulates sGC activity for the local regulation of CBF. Additionally, within the neuron itself, NO can exert both pre-synaptic and post-synaptic effects on the efficiency of neurotransmitter signaling. For example by increasing neurotransmitter release pre-synaptically, or by increasing the neurotransmitter receptor occupancy in the post-synaptic membrane. Furthermore, NO-sGC-cGMP signaling can also strengthen the synapse through PKG-mediated protein synthesis. BDNF: brain-derived neurotrophic factor, CaMKII: Ca^{2+}/calmodulin dependent protein kinase II, cGMP: cyclic guanosine monophosphate, EC: endothelial cell, nNOS: neuronal nitric oxide synthase, NO: nitric oxide, sGC: soluble guanylyl cyclase, PKG: protein kinase G, VASP: vasodilator-stimulated phosphoprotein, VSMC: vascular smooth muscle cell.

conditions is currently rather limited. The available evidence cumulatively suggests an important role of the NO-sGC-cGMP signaling axis in regulating the homeostasis and healthy function of the individual cells comprising the NVU. However, since most of these data derive from studies primarily focusing on pathological states, caution should be used when extrapolating such data to understand the physiological functioning of the NO-sGC-cGMP axis in the NVU.

1.5. The role of NO in sGC-cGMP signaling: maintaining a healthy balance

While the above sections primarily focus on the role of sGC and downstream cGMP effects in physiological NO-sGC-cGMP signaling, it is also important to take into account the effects of the upstream component, namely NO. Mechanistically, Ghosh et al., discovered that the insertion of heme into the sGC-β1 subunit is driven by 90 kDa heat shock protein (hsp90) [98], a process they later found to be NO-driven [99]. In its heme-free state (apo-sGC-β1), the sGC-β1 subunit does not associate with the α-subunit, thus does not form an active sGC heterodimer. Instead, apo-sGCβ1 associates with hsp90. For maturation of the Fe(II) sGC αβ-heterodimer, GAPDH delivers heme to apo-sGC-β1, facilitating heme-insertion into the β1-subunit through Hsp90-mediated processes (for an in-depth review, see [100]). Interestingly, it was found that low levels of NO can initiate GAPDH-dependent heme reallocation to apo-sGC-β1 [101]. Furthermore, Ghosh et al., [102] recently discovered that low endogenous levels of NO were also required for heme insertion in eNOS, nNOS and iNOS, which are also heme-dependent. In contrast, high levels of NO were found to dissociate the sGCαβ-heterodimer [103], and inhibited heme-insertion into NOS [102]. These findings were corroborated by Dao et al., [104] who demonstrated that chronically elevated NO lead to the degradation of Fe(II)sGC, but not apo-sGC. It is important to note that physiologically, the demarcation between 'low' and 'high' NO levels may be subtle, and a carefully maintained balance of NO levels is essential for physiological NO-sGC-cGMP signaling. However, in disease processes such as VCI, this balance is disrupted, leading to potentially detrimental effects on the NO-sGC-cGMP axis. These disease processes and their effects will be discussed in greater detail in Section 2.

2. NO-sGC-cGMP signaling: neurovascular components in VCI

This section will start with a brief overview of the main pathobiological processes in VCI, followed by information on the specific involvement of NO-sGC-cGMP signaling. VCI pathophysiology is associated with cerebral hypoperfusion, e.g. due to a reduced CBF, white matter lesions, vascular stiffening, and/or reduced BBB integrity (Fig. 3). Given the critical role of NO-sGC-cGMP signaling in regulating hemodynamics and BBB integrity under physiological conditions, any disruption of this pathway could be a significant pathological mechanism in VCI (Fig. 3). One important aspect of dysfunctional sGC signaling in this context revolves around oxidative stress. sGC is sensitive to oxidation by free radicals such as O_2^- (superoxide anion) and $ONOO^-$ (peroxynitrite), resulting in an oxidized state (Fe(III)sGC) or a heme-free apo-sGC state, which is inactive [30,32,33,36]. Additionally, oxidative stress can limit sGC activation by reducing the bioavailability of NO. The enzyme superoxide dismutase (SOD) catalyzes the

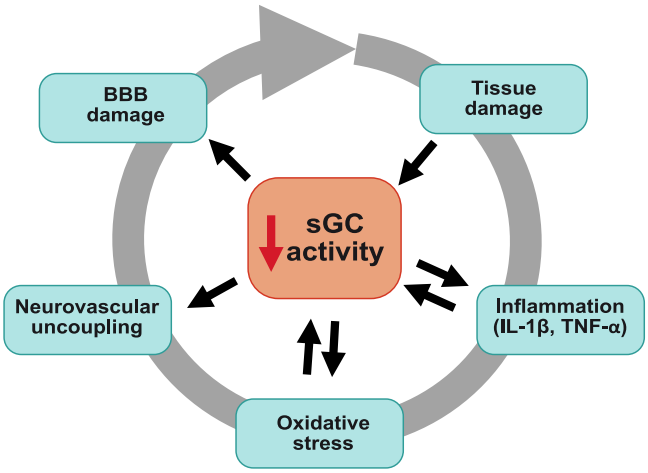

Fig. 3. Schematic representation of the vicious cycle of the main pathological mechanisms of VCI, with a central role for sGC. Based on the current literature, this hypothetical schematic of a vicious circle could be designed, in which the role of sGC is highlighted specifically. BBB: blood-brain barrier, IL-1β: interleukin-1β, sGC: soluble guanylyl cyclase, TNF-α: tumor necrosis factor α.

conversion of O_2^- into O_2 and H_2O_2, thereby preventing reactive damage by O_2^-. Under physiological conditions, the O_2^- concentration is low, thus physiological levels of NO can be maintained. However, under oxidative stress conditions, O_2^- levels rise, requiring a higher NO consumption. While the reaction between O_2^- and NO is very rapid, the reaction between NO and sGC is considerably slower due to the enzyme kinetics of sGC [105]. As a result, increased oxidative stress could reduce the bioavailability of NO for the slower reaction with sGC.

Other oxidative stress-related effects may involve eNOS uncoupling, where eNOS produces O_2^- by eNOS instead of NO [106–108]. This can lead to oxidative stress, increasing the risk for sGC oxidation and heme loss, which further decreases sGC activity. Additionally, eNOS uncoupling decreases NO bioavailability, which can also lead to reduced sGC activity. Importantly, eNOS uncoupling is considered a major mechanism underlying cerebrovascular endothelial dysfunction in VCI [109]. This endothelial dysfunction has been shown to lead to other pathological processes in VCI, including reduced BBB integrity and neurovascular uncoupling [109,110], and white matter damage [111]. As touched upon above, tight junctions between adjacent endothelial cells plays a crucial role in determining endothelial permeability, which is vital for BBB integrity [112,113]. Within neurodegenerative and neuroinflammatory states, dysfunctional endothelial processes seem to reduce tight junction integrity and impair paracellular transport systems [114]. Moreover, endothelial dysfunction can impair CBF as a result of neurovascular uncoupling. As mentioned earlier, NO plays a role in regulating vascular tone and CBF, and reduced NO bioavailability through eNOS uncoupling could therefore also lead to neurovascular uncoupling. A study by Toth et al. [115] demonstrated that both genetic eNOS depletion via an eNOS knockout, and pharmacological inhibition of NO synthesis lead to a decreased cortical CBF response. Many of the above mechanisms come together in a recent study by Neves et al. [116], which focused on Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL). CADASIL is a hereditary cSVD sub-type characterized by vascular dysfunction that predisposes for stroke and dementia. In their study, Neves et al. [116] discovered that patients with CADASIL suffered from oxidative damage of cerebral vessels. Furthermore, sGC oxidation was increased, and sGC activity and cGMP levels were decreased in patient-derived VSMCs. This study underscores the substantial contributions of impaired NO-sGC-cGMP signaling to cerebrovascular diseases such as cSVD and VCI. However, Chow et al., [117] revealed that neurovascular coupling is also regulated by an eNOS/NO-independent mechanism via caveolae within arteriolar endothelial cells. Thus, while neurovascular uncoupling could be a result of impairments within eNOS signaling, other mechanisms may also be involved.

In the context of sGC regulation, post-translational modifications also play a role. S-nitrosylation of sGC, a process in which the cysteine thiol side chain reacts with nitrogen species such as NO and $ONOO^-$ to form covalent S-nitrosothiol (SNO) groups [118], has been identified as a mechanism that can desensitize the enzyme [119]. Multiple additional S-nitrosylation sites have been discovered, including two sites in the catalytic domain of sGC that could directly impair sGC activity [120]. Elevated S-nitrosylation of sGC may occur due to eNOS overexpression and subsequent overproduction of NO [121]. Furthermore, S-nitrosylation could be linked to vascular dysfunction under oxidative stress and nitrosative stress conditions [122]. Additionally, oxidative stress has been shown to affect the stability and alternative splicing of sGC subunit mRNA, potentially reducing sGC availability [123].

Beyond the direct effects of oxidative stress, pro-inflammatory conditions are also related to sGC activity in VCI pathology. Hypoxia-induced tissue damage can trigger a pro-inflammatory state [124,125] and the activity and expression of sGC can be reduced by exposure to pro-inflammatory cytokines [126]. In fact, the production of pro-inflammatory cytokines such as TNFα and IL-1β can induce BBB damage [109,124]. This damage, in turn, leads to the production of reactive oxygen species, contributing to oxidative stress and endothelial dysfunction. Vice versa, oxidative and nitrosative stress can induce a pro-inflammatory response in endothelial cells [127]. Taken together, the different pathological processes associated with VCI appear to create a vicious circle of neurovascular dysregulation and cerebrovascular damage (see Fig. 3 for a schematic representation).

3. Direct targeting of sGC-cGMP signaling for the treatment of VCI

To adequately treat VCI, it may be imperative to disrupt the vicious cycle of cerebrovascular pathology (Fig. 3). The decrease in sGC activity caused by factors such as oxidative stress, eNOS uncoupling, nitrosative stress, and inflammation could exacerbate pathological processes such as endothelial dysfunction, a compromised BBB, and neurovascular uncoupling. Indeed, inhibition of sGC with selective sGC inhibitor 1 H-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one (ODQ) was found to potentiate oxidative stress-induced cell death in primary cortical neurons. Importantly, this effect could be reversed by the application of 8-Br-cGMP, a synthetic analog of cGMP [128]. These findings suggest that restoring the functionality of the NO-sGC-cGMP pathway may directly benefit the reversal of pathologies associated with oxidative stress in neurons and likely other cell types. In the following sections, the protective effects of restoring NO-sGC-cGMP signaling within and between different cell types will be discussed, with a focus on sGC as a potential novel target for VCI treatment.

3.1. *Targeting sGC: sGC activators and stimulators*

Currently, there are two distinct pharmacological approaches for targeting sGC to enhance its activity and consequently increase cGMP production (as illustrated in Fig. 4): either by stimulating the activity of Fe(II)sGC or by activating the activity of Fe(III)sGC and apo-sGC [129,130]. sGC stimulators act on Fe(II)sGC both independently of and in synergy with NO (Fig. 4). These stimulators bind and stimulate sGC without the need for NO, resulting in NO-independent cGMP production. Additionally, they can sensitize sGC to endogenous NO by stabilizing the NO-sGC binding. Thereby compensating for reduced NO availability and lower NO levels. The sGC activators have a different mode of action; they can enhance sGC activity in a heme-independent manner by binding to the unoccupied H-NOX domain [131]. Consequently, sGC activators can activate the inactive Fe(III)sGC/apo-sGC, thereby compensating for increased oxidation of sGC (Fig. 4).

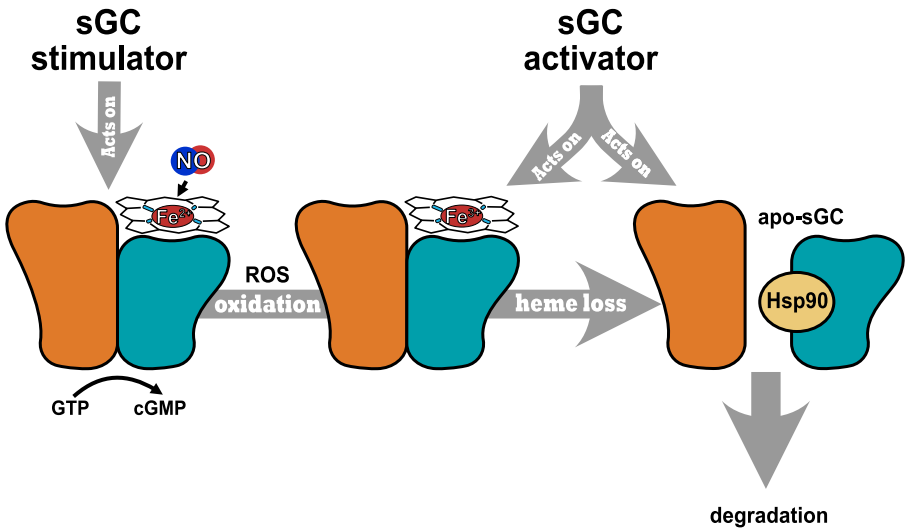

Fig. 4. The different heme-states of sGC and sGC agonist targets. sGC stimulators bind to the NO-sensitive and enzymatically active Fe(II)sGC. Upon oxidation (e.g. by reactive oxygen species (ROS)), conversion into Fe(III)sGC can occur, which is prone to full heme loss (apo-sGC). Both Fe(III)sGC and apo-sGC are enzymatically inactive due to their insensitivity to NO. sGC activators act heme-independently and therefore target specifically the Fe(III)sGC and apo-sGC.

For the treatment of cardiovascular disease, several sGC stimulators and activators have either been approved for clinical use or are currently being tested in phase I, II and III clinical trials. For example, riociguat (Adempas™) is the first in class sGC stimulator and was approved in 2013 for pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. Additionally, the sGC stimulator vericiguat (Verquvo®) was approved in 2021 for the treatment of chronic heart failure for patients with reduced ejection fraction. Ongoing clinical investigations are exploring sGC stimulators or activators for the treatment of various cardiovascular conditions, including chronic kidney disease, sickle cell disease, and type 2 diabetes with hypertension. For the most recent overview of clinical developments, see [10,132].

Research focusing on sGC as a target for neuronal disorders and cerebrovascular diseases has been limited, likely due to the lack of brain penetrability of most sGC stimulators and activators. In the following sections, a summary of this research will be given and subdivided into three categories: 1) indirect evidence; 2) preclinical neuronal evidence; 3) clinical evidence.

3.1.1. Indirect evidence of sGC agonism as a treatment for VCI

Most research with sGC agonists was performed on cells and systems distinct from the CNS, resulting in predominantly indirect evidence suggesting sGC agonism as a potential treatment for VCI. This section will highlight research that holds potential relevance for the CNS or cerebral vasculature. This includes research outside the CNS that specifically demonstrates the effects of sGC agonism on pathological processes also present in VCI, such as inflammation and oxidative stress.

Vascular research has shown that sGC agonists can offer protection against oxidative stress. For instance, treatment with the sGC stimulator BAY 41–2272 was effective in preventing apoptosis induced by $ONOO^-$ in human monocyte-derived macrophages [133]. Moreover, aortic rings from rats treated in vivo with the sGC activator cinaciguat were protected against oxidative damage induced by *ex vivo* $ONOO^-$ application, along with improved endothelial functioning [134]. Similar effects were observed for both the stimulator BAY 41–2272 and the activator BAY 60–2770 *ex vivo* in isolated rat iliac arteries lacking endothelium and exposed to $ONOO^-$ [135]. Furthermore, the sGC activator runcaciguat was able to activate sGC [136] and increase renal blood flow even after the treatment with sGC inhibitor ODQ [137]. Under sGC desensitization/oxidation conditions induced by in vivo chronic nitroglycerin treatment, the sGC activator BAY 60–2770 was able to restore endothelial function, reduce oxidative stress, and induce vasodilation. In contrast, the sGC stimulator BAY 41–8543 had limited beneficial effects both in vivo and *ex vivo* [138]. Interestingly, another study showed that sGC stimulator BAY 41–8543 was able to restore sGC-cGMP dependent vasodilation in vivo under conditions of reduced NO bioavailability due to the presence of NO scavengers [139]. These findings collectively suggest that both sGC stimulators and activators may be beneficial under oxidative stress conditions, and when NO availability is low, although the choice of sGC agonist may depend on the target mechanism of interest.

Moreover, sGC agonists have demonstrated anti-inflammatory properties. For example, the sGC stimulator IW-1973 exhibited anti-inflammatory effects in vivo in the Dahl salt-sensitive rat model for hypertension [140], and both the sGC stimulator BAY 41–2272 and sGC activator BAY 60–2770 protected against ischemia-induced inflammation in mice [141,142]. Most studies have investigated various sGC stimulators and activators in oxidative-stress or inflammation models within the cardiovascular, cardiopulmonary and renal (circulatory) system. This is reflected by the relatively high number of clinical trials exploring sGC stimulators and sGC activators in cardiovascular diseases and related areas [10]. Although encouraging, the implications of this cardiovascular evidence for the benefits of sGC modulation in the cerebrovascular system associated with VCI pathology, while rationally-based, remain indirect.

The sGC activator cinaciguat, originally developed for acute decompensated heart failure [10], was able to improve the coupling between the heart and the autonomic nervous system [143]. The authors suggested that cinaciguat could re-couple inter-organ communication pathways, although further research is needed. Nevertheless, it can be hypothesized that this concept might also extend to neurovascular coupling. In a study with BAY 41–2272, it was shown that sGC activation has vasodilatory effects on isolated rat basilar artery rings, which provides an indication that sGC activation affects the cerebral vasculature [144]. This implies that the cardiovascular protective effects associated with sGC agonists may also apply to the cerebrovascular system. However, it is essential to exercise caution, as not all literature immediately supports a solely positive effect of sGC agonism on VCI.

A study by Chow et al., [145] has indicated that in Alzheimer's Disease (AD), reduced CBF could be attributed to a hypercontractile state of the cerebral vasculature. This hypercontractility appears to be mediated by enhanced expression of serum response factor (SRF) and myocardin (MYOCD), in turn leading to an increase in contractile proteins in VSMCs. Interestingly, prior research has shown that PKG-I can

stimulate SRF-MYOCD-dependent gene expression [146], which may at first sight suggest that sGC agonism might exacerbate the already hypercontractile phenotype in AD. Furthermore, in a study by Bell et al., [147] it was shown that SRF-MYOCD-dependent gene expression in AD inhibited amyloid peptide clearance. This further argues against the use of sGC agonists for the treatment of vascular dysfunction in AD.

Nonetheless, a study by Boden et al., [148] has revealed that sGC stimulator vericiguat can reduce aortic stiffness. It is worth noting that in atherosclerotic plaques, VSMCs contribute to vascular pathology through phenotypic switching [149]. This phenotypic switch is accompanied by a loss in expression of contractile proteins, i.e. the VSMCs switch from a 'contractile' to a 'differentiated' phenotype [149,150]. While a healthy contractile phenotype allows VSMCs to regulate factors such as blood flow, vessel diameter and blood pressure, the phenotypic switch contributes to vascular stiffening [150], and intimal hyperplasia [151]. Furthermore, it has been shown that NO promotes the expression of contractile proteins in VSMCs [152], and helps maintain a contractile phenotype in a cGMP-independent manner [153]. At the same time, NO is also a well-known vasorelaxant in arterioles and capillaries, and regulates blood flow directly in a cGMP-dependent manner, which is in turn independent from VSMC protein expression. Altogether, this shows that the NO can induce both vasorelaxation and enhanced expression of contractile proteins in VSMCs in a physiological state, while the maintenance of a contractile VSMC phenotype is partially cGMP-independent. Thus, enhancing NO-signaling through sGC agonism may still hold promise for the treatment of cerebrovascular disease and cognitive impairments, even within an already hypercontractile phenotype such as seen in AD. Nevertheless, it remains important for future research to determine whether sGC agonism indeed positively impacts vascular health in AD and whether this, in turn, affects the symptomatology of AD.

3.1.2. Preclinical neuronal evidence of sGC agonism as a treatment for VCI

YC-1 was the first sGC stimulator to be thoroughly investigated for its memory-enhancing properties [154,155] and its impact on the neuronal and cerebrovascular systems is currently the most well-documented among all sGC agonists. YC-1 was able to attenuate the inflammatory effects in cerebral ischemia [156,157], and effectively prevented LPS-induced activation of iNOS in microglia [158]. Furthermore, YC-1 exhibited neuroprotective effects in both in vitro neuronal ischemia and in vivo ischemic conditions [159]. However, the effects of YC-1 must be interpreted with care, since it is also known to inhibit hypoxia-inducible factor 1α [160], whose activity seems to inhibit BBB damage repair [161]. While research on sGC agonists in the CNS and cerebrovascular system is limited, available literature already indicates that sGC stimulators can enhance cGMP production in neurons [162], influence short-term synaptic plasticity [163], and improve memory performance through peripheral mechanisms [164]. Furthermore, sGC activators have been shown to have neuroprotective properties after stroke [165].

Within the cerebrovascular research field, the majority of studies so far have primarily focused on sGC activation in stroke [165]. While most sGC agonists typically do not penetrate the brain under physiological conditions, neurovascular disorders such as VCI are frequently associated with increased BBB permeability. Consequently, it is somewhat surprising that, to our knowledge, there are very few studies attempting to target sGC for the treatment of cognitive or neurovascular problems (e.g. see [154,164–167]). Indeed, a study investigating the memory enhancing effects of vericiguat showed that despite its inability to penetrate the brain, vericiguat was capable of enhancing memory performance in rats without affecting overall CBF [164]. However, it is worth noting that this study did not directly measure the microvasculature (e.g. by laser speckle contrast imaging). Thus, it cannot be excluded that changes in CBF were induced locally, resulting in improved memory performance. In a study by Correia et al. [168], it was found that vericiguat induced a change in the fMRI BOLD signal in cortical areas in rats, albeit to a lesser extent compared to the fMRI BOLD signal change induced by the brain-penetrant sGC stimulator zagociguat (CY6463, formerly known as IW-6463). The difference between zagociguat and vericiguat can likely be attributed to the fact that vericiguat cannot penetrate the brain, and thus cannot stimulate neuronal activity in deeper cortical areas. It can therefore be hypothesized that vericiguat enhanced memory in rats through a localized change in CBF within the microvasculature, thereby indirectly enhancing neuronal activity in the immediate surrounding areas. Such subtle local effects are often not detectable through methods such as fMRI BOLD, and would require validation via a more sensitive approach. Nevertheless, whether the inability of vericiguat to penetrate the brain would impact its clinical effectiveness, particularly in the presence of pathologically increased BBB permeability, remains to be investigated.

So far, only three studies have identified sGC agonists with the capability to cross the BBB. A first rodent study investigated the effects of sGC stimulator zagociguat on cerebrovascular function and memory enhancement. Zagociguat was reported to enhance memory performance in rodents through central mechanisms and also improved cerebrovascular circulation [168]. A second study introduced the brain-penetrant sGC stimulator CYR119, which was found to enhance CREB phosphorylation, a marker for neuroplasticity, in vitro in primary rat hippocampal neurons. Additionally, CYR119 attenuated the LPS-induced increase in proinflammatory markers in vitro in the SIM-A9 microglial cell line, and reduced proinflammatory markers in vivo in diet-induced obese mice and quinolinic acid-treated rats as models for neuroinflammation. In the dorsal striatum of quinolinic acid-treated rats, the anti-inflammatory effect of CYR119 treatment could be related to CREB phosphorylation [169]. A third rodent study described brain-penetrant sGC stimulator BAY-747 and sGC activator runcaciguat, investigating their effects on long-term and short-term memory in rats, along with their effects on plasticity markers in the mouse hippocampus [170]. Both BAY-747 and runcaciguat were reported to enhance long-term memory, although their underlying central mechanisms appeared to be distinct. The activator runcaciguat seemed to act stronger on the glutamatergic system, while the stimulator BAY-747 appeared to act more on the neurotrophic system. It is important to note that this study only explored the central mechanisms for a single dose of BAY-747 and runcaciguat. Differences in bioavailability, BBB penetration rates, actual drug concentrations within the CNS, and potential variations in interaction dynamics between the sGC enzyme and the stimulator/-activator were not fully considered. These potential kinetic differences should be more thoroughly investigated in order to draw definitive conclusions about the central mechanisms related to the effects of stimulators and activators within the CNS. Collectively, the three studies mentioned above still indicate that brain-penetrant sGC agonists can induce neuroplasticity related to cognitive enhancement, improve cerebrovascular circulation, and act to reduce inflammation. Nevertheless, the above data seem to globally offer a promising rationale for the use of sGC agonists in treating neurodegenerative diseases characterized by cognitive, neuroplastic, cerebrovascular, and inflammatory pathologies, such as in VCI.

3.1.3. Clinical evidence of sGC agonism as a treatment for VCI

A clinical study using riociguat to attenuate biperiden-induced memory impairments in healthy subjects proved unsuccessful [166]. This outcome may have been attributed to the limited brain penetrability, thus making it difficult to directly target neurons. Additionally, the optimal dose for potential beneficial peripheral, cerebrovascular effects that could enhance memory may have been missed due to its potential interaction with blood pressure regulation. To our knowledge, only one other clinical study has been conducted with sGC agonists for neurodegenerative diseases. According to a press-release from Cyclerion Therapeutics Inc., a phase 1 study indicated that the brain-penetrant sGC stimulator zagociguat had a positive impact on EEG alpha power, improved the N200 auditory event-related potential, and showed

positive effects on an objective saccadic eye movement task. These findings collectively suggest that zagociguat increased cognitive and attention processing [171]. Notably, no effects of zagociguat on CBF were observed. Similarly, zagociguat was reported to be effective in a phase 2 study for the treatment of cognitive impairments associated with schizophrenia (CIAS), according to a recent press release [172]. A phase 2 study with zagociguat as a treatment for (AD) with vascular pathology was ongoing (NCT04798989), but has recently been terminated due to "enrollment issues". It is expected that results from this study, with a reduced sample size, will be available soon and could provide relevant insights into the effects of sGC stimulation on VCI-related dementias. Interestingly, Cyclerion reported in a 2020 press-release that zagociguat enhances cognition in elderly, without affecting CBF [171]. Two recent publications by Van Kraaij et al., indicate that zagociguat is safe and well-tolerated in healthy elderly [173,174]. Additionally, they show that zagociguat does not alter CBF or brain metabolite concentrations at a dose of 15 mg for 14 consecutive days; nor is a clear cognition enhancing effect reported [173]. The cognition enhancing effects announced in the 2020 press release [171], are seemingly not published (yet). Interestingly, positive results from a cognitive test battery have been announced for the treatment of CIAS at a tested dose of 15 mg for 14 consecutive days [172], yet no results on CBF were reported in this 2022 press-release by Cyclerion. In another 2022 press-release, Cyclerion reported increased CBF after treatment with 15 mg zagociguat for 29 consecutive days in an open-label study for the treatment of Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) [175]. This suggests that, while 14-day treatment with 15 mg zagociguat did not alter CBF in elderly, more prolonged treatment might still increase CBF. Therefore, it cannot be excluded that the effects of 15 mg zagociguat are – at least in part – CBF mediated, especially after longer treatment periods. It is important to note that the MELAS study was performed in a younger population, thus age may also be a factor in the effects of zagociguat on CBF. Unfortunately, the effects of zagociguat in a standardized cognitive test battery have not yet been fully published. Therefore, the future results of the aforementioned phase 2 study with zagociguat as a treatment for AD with vascular pathology (NCT04798989) may shed important light on the clinical cognition enhancing mechanisms of sGC stimulators in the CNS, particularly in regard to whether neuronal and vascular (e.g. CBF) effects act synergistically.

3.1.4. *The future of sGC agonism for the treatment of VCI: sGC stimulators, activators, or both?*

While the difference between the mechanisms of action of sGC stimulators and activators is well-described in literature (Fig. 4), the question of which agonist is 'best' for the treatment of VCI remains unanswered. In addition to the specific physicochemical and pharmacokinetic differences for each individual molecule, there might also be mechanistic differences between these two classes of compounds. Indeed, VCI encompasses various diseases, including post-stroke dementia, and mixed dementias such as AD with vascular pathology [3]. Therefore, determining "which agonist is best" may not be clear-cut and could be disease-specific or even patient-specific. In the following paragraph, a few studies will be highlighted to discuss the potential choice between an sGC stimulator or activator for different VCI subtypes.

In literature, the dynamics of NO-sGC-cGMP signaling during ischemic stroke have been clearly described in a study by Langhauser et al., [165] which shows that Fe(II)sGC is almost fully absent after ischemic stroke, in contrast to apo-sGC. This suggests that sGC activators would be most effective for the immediate treatment of ischemic stroke, in comparison to sGC stimulators. However, it could be hypothesized that after an initial boost of sGC-dependent cGMP signaling via an sGC activator, levels of Fe(II)sGC may rise, and the patient might benefit more from long-term treatment with an sGC stimulator to sustain the rescue of the NO-sGC-cGMP system. In AD, sGC research remains relatively limited. Nevertheless, one study found a reduced expression of the sGC β1-subunit in astrocytes of AD patients [176]. This could suggest that an sGC activator, which binds to the α-subunit even in the absence of the β1-subunit, would be the preferred agonist for treatment of AD. Another study used the Swedish-Arctic transgenic AD mouse model to investigate vascular dysfunction in AD. This mouse model is characterized by rapid plaque formation at a very young age, and some mice develop severe cerebral amyloid angiopathy (CAA) as a vascular pathology. Interestingly, this study concluded that the vascular dysfunction in that model could be attributed to a reduced NO availability, and is not a direct consequence of CAA [177]. Based on this study, it could be hypothesized that an sGC stimulator would be the preferred treatment strategy.

Studying VCI subtypes is often limited by translational hurdles in the available models and methods, which can lead to contradictory findings in the "stimulator versus activator" debate. Furthermore, there numerous subtypes, adding complexity to the issue. The choice of sGC agonist is unlikely to be generalized for any given VCI subtype and may depend on different phenotypes within each subtype. It can even be speculated that treatment decisions should be entirely patient-specific. For instance, the hypercontractile phenotype found in AD [145,147] might contraindicate the use of sGC agonists. Additionally, patient- or disease-specific NO levels could be indicative of the choice between a stimulator or an activator: patients with low endogenous NO levels may benefit more from an sGC stimulator, whereas for patients with higher NO levels an activator may be preferred. Perhaps, the rapid advancements within the field of induced pluripotent stem cells (iPSCs), will enable the development of future patient-derived models (e.g. of the BBB) for studying and determining the optimal treatment strategy for different VCI subtypes, and perhaps individual patient-derived models may even be feasible for more personalized medicine. Additionally, future research could focus on biomarkers (e.g. SRF expression levels) to determine more personalized treatment strategies. Nevertheless, choosing between an sGC stimulator or activator may not be an "either-or" choice, and well-timed combination treatments might offer the most benefit in treating VCI.

3.2. *Alternative ways of targeting cGMP signaling: PDE inhibitors*

As previously mentioned, PDEs break down cGMP and thereby regulate the rate of cGMP production. This means that the use of PDE inhibitors, particularly those targeting cGMP-specific PDEs (PDE5, 6, and 9) can increase the available cGMP levels. Given the known cognitive improvement associated with PDE inhibition [178,179], cGMP-specific PDEs may represent interesting targets in the treatment of VCI.

Most of the research in this area has centered on the PDE5 family in relation to VCI, and, to the best of our knowledge, no studies have explored the roles of PDE6 and PDE9 in the context of VCI. The PDE5 family is expressed in the small penetrating arteries of the brain, which is why its expression has been research focus in the study of patients suffering from cSVD, a contributing factor to VCI pathology. Interestingly, PDE5 expression levels were not found to be associated with age or degree of cSVD severity [180]. In addition to PDE5 expression, PDE5 inhibitors and their potential as a cSVD treatment were also investigated. The PDE5 inhibitor tadalafil was used in a small clinical pilot study focused on patients with lacunar stroke, which is another contributor to VCI pathology. A single dose of 20 mg of tadalafil significantly improved cerebral blood flow compared to a placebo-treated control group. Interestingly, it was found that tadalafil also lowered levels of the pro-inflammatory cytokine IL-1β [181]. Conversely, the PASTIS clinical trial, which studied tadalafil in older patients suffering from symptomatic cSVD, reported that the increase in cerebral blood flow was not significant compared to the placebo group [182]. However, it must be noted that both clinical trials had relatively low sample sizes, which could impact the different effects found

between the two clinical trials. Worth mentioning is a systematic review by Pauls et al. [183], which presented data where PDE5 inhibitors were specifically used to improve cerebral blood flow in several cerebrovascular pathologies. It was concluded that PDE5 inhibition increased the responsiveness of the cerebral blood vessels, and increased NO-cGMP signaling following PDE5 inhibition was mentioned as potential mode of action [183]. Additionally, a rodent study using a rat model for chronic cerebral hypoperfusion revealed that the PDE5 inhibitor sildenafil reduced hippocampal cell death following chronic cerebral hypoperfusion, although this did not prevent memory impairments [184].

Of note, the cGMP signaling effects enhanced by PDE inhibitors could also result from NO-sGC independent cGMP production. Therefore, any findings involving cGMP-enhancing PDE inhibitors in VCI or any other vascular or neuronal pathology cannot be directly extrapolated to NO-sGC-cGMP signaling pathways. Despite the promising results of PDE inhibitors on cerebral blood flow and memory, more comprehensive research is needed to determine whether cGMP-specific PDE inhibition could be a viable treatment for VCI and whether this is NO-sGC dependent. Ideally, future research should focus on a direct comparison between sGC agonists and cGMP-specific PDE inhibitors to elucidate the potential of PDE inhibitors as a treatment for VCI.

4. Concluding remarks and future perspectives

The physiological significance of the NO-sGC-cGMP pathway for both the CNS and the cardiovascular system cannot be overstated. Neurovascular coupling and the BBB collectively form the interface where the cardiovascular and neuronal NO-sGC-cGMP systems intersect and interconnect, and it is precisely at this interface where VCI pathology becomes evident. While most CNS and cardiovascular research into the molecular pathology focuses upstream of sGC at the NOS/NO level, evidence suggests that this neurovascular dysregulation can be both a consequence and a cause of altered sGC activity and expression through: (I) eNOS uncoupling and (II) NO scavenging leading to a reduced NO availability, and (III) oxidation of sGC and subsequent heme-loss. All this affects cellular sGC-cGMP signaling and can lead to specific dysfunctions.

Within the cardiopulmonary, cardiovascular and renal system, sGC agonists can restore physiological NO-sGC-cGMP signaling and have been clinically demonstrated to be an effective treatment for distinct diseases such as chronic heart failure and pulmonary hypertension. Two sGC stimulators, riociguat and vericiguat, have already been approved for cardiovascular indications, and more sGC stimulators and activators are currently being tested in clinical trials for a wide range of vascular indications. However, the effects of sGC agonists within the CNS and even the cerebrovascular system remain largely underexplored. sGC activators may hold promise for stroke treatment, while sGC stimulators appear to have potential in enhancing synaptic plasticity and memory.

Research that specifically targets the cardiovascular and neuronal systems, especially within the context of VCI pathology, is limited. Nevertheless, evidence primarily from cardiovascular disease pathologies indicates that modulating sGC may indeed be beneficial for the treatment of cerebrovascular pathology associated with VCI. Therefore, research focusing on the effects of sGC modulation within this pathology seems timely. Moreover, investigating the impact on brain plasticity through direct neuronal effects is crucial, including considering the potential peripheral (cardio)vascular side effects. In addition, the differential effects of sGC stimulation versus sGC activation under various pathological circumstances underscore the importance of identifying the precise underlying pathological mechanisms of different VCI endophenotypes and subsequently choosing the appropriate treatment, whether it involves an sGC stimulator, activator, or specific combination of the two. Based on what we know regarding a) the overall beneficial effects of sGC direct agonists in cardiovascular disease and b) the functional importance of the NO-sGC-cGMP axis in the neurovascular unit and in neurons themselves, it is reasonable to suggest that sGC agonists seem promising therapeutic agents in VCI, whose utility should be further investigated both at the preclinical and the clinical level.

Ethics approval and consent to participate

Not applicable.

CRediT authorship contribution statement

Ellis Nelissen: Conceptualization, Writing – original draft, Writing – review & editing, Investigation, Visualization. **Melissa Schepers:** Writing – original draft, Writing – review & editing, Visualization. **Laura Ponsaerts:** Writing – original draft. **Sébastien Foulquier:** Conceptualization, Writing – original draft, Writing – review & editing. **Annelies Bronckaers:** Writing – review & editing, Supervision. **Tim Vanmierlo:** Conceptualization, Writing – review & editing, Supervision. **Peter Sandner:** Conceptualization, Writing – review & editing, Investigation, Supervision. **Jos Prickaerts:** Writing – review & editing.

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JP has a proprietary interest in sGC agonists in the treatment of cognitive impairments. PS is a full time employee of Bayer AG, Pharmaceuticals. All other authors declare no conflict of interest.

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